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Antimicrobial Susceptibility of Vancomycin-Resistant *Leuconostoc*, *Pediococcus*, and *Lactobacillus* Species

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Eighty-five strains of vancomycin-resistant gram-positive bacteria from three genera, *Leuconostoc*, *Pediococcus*, and *Lactobacillus*, were tested to determine susceptibility to 24 antimicrobial agents by broth microdilution and to 10 agents by disk diffusion. The MICs of vancomycin and teicoplanin ranged from 64 to >512 μg/ml; however, the MICs of daptomycin, a new lipopeptide, were all ≤0.25 μg/ml. None of the organisms were resistant to imipenem, minocycline, chloramphenicol, gentamicin, or daptomycin. The MICs of penicillin were in the moderately susceptible range for all but three strains. Susceptibility to the other agents varied by genus and, in some cases, by species. When disk diffusion results were compared with MICs for drugs recommended for streptococci by the National Committee for Clinical Laboratory Standards, Villanova, Pa., few very major or major errors were obtained, but the number of minor errors was 19.3%. Therefore, we recommend that MIC testing be used instead of disk diffusion testing for these organisms.

Because of the increase in nosocomial infections caused by gram-positive cocci, especially staphylococci (both *Staphylococcus aureus* and coagulase-negative staphylococci) (T. Horan, D. Culver, W. Jarvis, G. Emori, S. Banerjee, W. Martone, and C. Thornsberry, Antimicrob. Newsl. 5:65–67, 1988), and because of the growing prevalence of methicillin resistance in staphylococci (9), vancomycin has been used more often for treating patients who have or who are suspected of having infections caused by gram-positive organisms. Reports of clinical infections caused by vancomycin-resistant organisms have been more frequent in recent years, with resistance in staphylococci (22), enterococci (14–16, 26), and lactobacilli (1, 8, 11) described. Clinically significant vancomycin resistance in *Leuconostoc* and *Pediococcus* spp. was rarely reported before 1985 (19). The first case of a clinically significant infection caused by a *Leuconostoc* sp. was reported as being caused by a *Streptococcus sanguis* II strain in 1984 (24). The identity of the strain reported was later questioned (C. Thornsberry and R. Facklam, Antimicrob. Newsl. 1:63–64, 1984) and reidentified as *Leuconostoc* sp. by one of us (R.R.F.).

Since then, a number of clinically significant infections caused by *Leuconostoc* spp. have been reported (2, 3, 5, 10, 12, 13, 19–21, 27), including a case of meningitis in a previously healthy 16-year-old girl (4). There has been only one report of infections caused by *Pediococcus* sp. (3), although many such strains from clinical sources have been submitted to the Centers for Disease Control for identification or antimicrobial susceptibility studies (7). Vancomycin resistance in lactobacilli has also been reported (1, 8, 11), but resistance of and clinical infection caused by *Lactobacillus confusus*, an organism that may often be confused with these gram-positive cocci, have not been documented.

Because of the possibility that these organisms may be pathogens, we tested a number of them to determine patterns of antimicrobial susceptibility and the suitability of the disk diffusion test for predicting that susceptibility.

MATERIALS AND METHODS

Bacterial strains. Seventy-nine clinical isolates and six type strains, identified by methods described by Facklam et al. (7), were included in the study. The clinical isolates tested were *Leuconostoc mesenteroides* (n = 18), *L. citreum* (n = 12), *L. pseudomesenteroides* (n = 4), *L. lactis* (n = 2), *Leuconostoc* sp. (n = 7), *Pediococcus acidilactici* (n = 20), *P. pentosaceus* (n = 3), *Lactobacillus confusus* (n = 11), and *Lactobacillus* sp. (n = 2). The type strains included were *L. mesenteroides* ATCC 8293, *L. lactis* ATCC 19256, *L. dextranicum* ATCC 19255, *L. paramesenteroides* ATCC 33313, and *P. pentosaceus* SS947 and SS1107. The sources of the strains were blood (n = 55), urine (n = 2), peritoneal fluid (n = 4), wounds (n = 4), abscesses (n = 4), cerebrospinal fluid (n = 3), a stool specimen (n = 1), a lung biopsy (n = 1), unknown (n = 5), and stock cultures (n = 6).

Susceptibility testing. The antimicrobial agents tested by broth microdilution were received from the manufacturers as powders suitable for susceptibility testing and are listed in Table 1. For disk diffusion, the antimicrobial agents were oxacillin and ciprofloxacin in addition to those recommended by the National Committee for Clinical Laboratory Standards (NCCLS), Villanova, Pa. (17), for nonenterococcal streptococci.

Before beginning the study, seven strains (three leuconostocs, three pediococci, and one lactobacillus) were tested for growth in nine different broth bases (Mueller-Hinton, Schaedler, heart infusion, brain heart infusion, brucella, anaerobe [experimental], Trypticase soy broth, Columbia, and *Haemophilus* test media; data not shown), both unsupplemented and supplemented with 5% lysed horse blood, 5% lysed rabbit blood, 3% horse serum, and 3% rabbit serum and incubated in both air and 5% CO_2.

MICs were determined by broth microdilution (18), using cation-supplemented Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) with 5% lysed horse blood. For disk diffusion testing, commercially prepared Mueller-Hinton agar with 5% sheep blood (BBL Microbiology Systems,

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TABLE 1. MICs and percentages of strains that are susceptible to 32 antimicrobial agents

Drug	Organism[a]	MIC (μg/ml)[b]			Breakpoint concn (μg/ml)[c]	% Susceptible
		Range	50%	90%		
Vancomycin	Leuconostoc	256–>256	>256	>256	≤4	0
	Pediococcus	256–>256	>256	>256		0
	Lactobacillus	>256	>256	>256		0
Teicoplanin	Leuconostoc	128–>256	>256	>256	≤4[d]	0
	Pediococcus	64–>256	>256	>256		0
	Lactobacillus	128–>256	>256	>256		0
Daptomycin	Leuconostoc	≤0.25	≤0.25	≤0.25	≤2[d]	100
	Pediococcus	≤0.25–0.5	≤0.25	≤0.25		100
	Lactobacillus	≤0.25	≤0.25	≤0.25		100
Penicillin	Leuconostoc	0.03–2	0.5	1	≤0.12	6
	Pediococcus	0.5–2	0.5	1		0
	Lactobacillus	0.25–2	0.5	1		0
Ampicillin	Leuconostoc	0.03–2	1	2	≤0.12	2
	Pediococcus	1–4	2	4		0
	Lactobacillus	0.25–1	0.5	1		0
Cephalothin	Leuconostoc	0.12–32	4	16	≤8	87
	Pediococcus	2–16	4	16		79
	Lactobacillus	8–16	8	16		77
Cefaclor	Leuconostoc	2–64	16	32	≤8	30
	Pediococcus	32–128	32	64		0
	Lactobacillus	16–128	128	128		0
Cefamandole	Leuconostoc	0.5–64	16	32	≤8	49
	Pediococcus	8–16	16	16		42
	Lactobacillus	4–8	8	8		100
Cefuroxime	Leuconostoc	≤0.25–32	8	16	≤8	66
	Pediococcus	4–16	8	8		88
	Lactobacillus	2–>128	32	>128		23
Ceftizoxime	Leuconostoc	≤0.25–128	8	32	≤8	62
	Pediococcus	≤0.25–32	8	16		50
	Lactobacillus	4–>128	16	>128		31
Ceftriaxone	Leuconostoc	≤0.25–128	8	32	≤8	57
	Pediococcus	≤0.25–16	16	16		38
	Lactobacillus	1–>128	16	128		31
Cefotaxime	Leuconostoc	≤0.25–64	8	16	≤8	66
	Pediococcus	2–16	8	8		88
	Lactobacillus	1–>128	16	>128		31
Ceftazidime	Leuconostoc	4–>128	64	128	≤8	17
	Pediococcus	16–64	32	64		0
	Lactobacillus	4–>128	>128	>128		7
Imipenem	Leuconostoc	≤0.06–8	2	8	≤4	81
	Pediococcus	≤0.06–0.12	0.12	0.12		100
	Lactobacillus	≤0.06–0.12	≤0.06	0.12		100
Erythromycin	Leuconostoc	≤0.015–0.06	0.03	0.06	≤0.5	100
	Pediococcus	≤0.015–>128	0.03	0.06		92
	Lactobacillus	0.03–0.06	0.06	0.06		100
Roxithromycin	Leuconostoc	≤0.06–0.25	0.12	0.12		
	Pediococcus	≤0.06–>16	≤0.06	0.12		
	Lactobacillus	≤0.06–0.12	≤0.06	0.12		
Josamycin	Leuconostoc	≤0.06–0.25	0.12	0.25		
	Pediococcus	≤0.06–32	0.25	0.25		
	Lactobacillus	≤0.06–0.25	0.12	0.12		

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TABLE 1—*Continued*

Drug	Organism[a]	MIC (μg/ml)[b]			Breakpoint concn (μg/ml)[c]	% Susceptible
		Range	50%	90%		
Clindamycin	Leuconostoc	≤0.008–2	0.015	0.06	≤0.5	98
	Pediococcus	≤0.008–32	0.015	0.015		96
	Lactobacillus	≤0.008–0.12	0.03	0.06		100
Gentamicin	Leuconostoc	≤0.25–0.5	≤0.25	0.5	≤4	100
	Pediococcus	0.5–4	1	2		100
	Lactobacillus	≤0.25–1	≤0.25	0.5		100
Tobramycin	Leuconostoc	≤0.25–2	0.5	1	≤4	100
	Pediococcus	2–16	4	8		67
	Lactobacillus	0.5–4	0.5	4		100
Streptomycin	Leuconostoc	0.5–8	2	8	≤8[d]	100
	Pediococcus	4–2000	16	32		29
	Lactobacillus	2–16	4	8		92
Kanamycin	Leuconostoc	1–16	4	16	≤16	100
	Pediococcus	8–64	32	64		29
	Lactobacillus	2–32	4	16		92
Tetracycline	Leuconostoc	0.5–16	4	8	≤4	77
	Pediococcus	4–64	16	32		4
	Lactobacillus	4–32	16	16		15
Doxycycline	Leuconostoc	0.25–16	4	8	≤4[d]	91
	Pediococcus	4–16	8	16		29
	Lactobacillus	2–16	8	8		38
Minocycline	Leuconostoc	0.25–4	1	2	≤4[d]	100
	Pediococcus	2–8	4	8		75
	Lactobacillus	0.5–2	2	2		100
Chloramphenicol	Leuconostoc	2–16	4	8	≤8	98
	Pediococcus	1–8	2	4		100
	Lactobacillus	2–8	8	8		100
Thiamphenicol	Leuconostoc	2–16	8	16		
	Pediococcus	2–16	8	8		
	Lactobacillus	4–16	8	16		
Rifampin	Leuconostoc	0.06–64	1	8	≤1	55
	Pediococcus	0.12–4	0.5	2		88
	Lactobacillus	16–32	32	32		0
Ciprofloxacin	Leuconostoc	0.5–4	2	4	≤1	24
	Pediococcus	2–16	8	16		0
	Lactobacillus	1–4	2	4		38
Sulfamethoxazole	Leuconostoc	64–>512	>512	>512	≤256	11
	Pediococcus	>512	>512	>512		0
	Lactobacillus	>512	>512	>512		0
Trimethoprim	Leuconostoc	≤0.5–16	4	8	≤8	98
	Pediococcus	2–16	8	8		96
	Lactobacillus	8–32	16	32		23
Trimethoprim-sulfamethoxazole	Leuconostoc	0.03–16	1	4	≤2	70
	Pediococcus	0.12–8	2	8		54
	Lactobacillus	4–>16	16	>16		0

[a] Numbers of organisms tested: *Leuconostoc* spp., 47; *Pediococcus* spp., 24; and *Lactobacillus* spp., 13.
[b] 50% and 90%, MIC for 50 and 90% of isolates, respectively.
[c] Breakpoints used are the susceptible ones defined by the NCCLS (18). Where no breakpoint is indicated, one was not found.
[d] Breakpoint not found in NCCLS M7 (18).

Cockeysville, Md.) was used. Inoculum was prepared for both tests by suspending cells grown on an overnight blood agar plate into Mueller-Hinton broth. The inoculum was first adjusted to a 0.5 McFarland standard to inoculate the disk diffusion plates and then further adjusted to a 1.0 McFarland standard and appropriately diluted to inoculate the broth microdilution plates by using the MIC 2000 mechanical inoculator (Dynatech Laboratories, Inc., Chantilly, Va.).

The final inoculum for the MIC test was 1×10^5 to 5×10^5 CFU/ml. MIC plates were incubated in ambient air, sealed in plastic bags. Disk diffusion plates were incubated in both ambient air and 5% CO_2. All plates were incubated at 35°C for 20 to 24 h.

β-Lactamase testing was performed by using a nitrocefin solution at a concentration of 500 μg/ml. Samples were suspended in 0.05 ml of the nitrocefin solution and read for a color change for up to 60 min. Tests for chloramphenicol acetyltransferase production were performed with a commercially available disk test (Remel, Lenexa, Kans.).

RESULTS

None of the media tested grew the strains better than cation-supplemented Mueller-Hinton broth with 5% lysed horse blood, the medium we prefer for testing fastidious organisms. Additional CO_2 was not needed for the broth testing. However, one strain of *P. acidilactici* would not grow in the broth microdilution plates and therefore is not included in the study. The majority of the strains grew much better on agar medium if incubated in 5% CO_2; therefore, only zone diameters obtained in CO_2 are considered in this report.

In Table 1, the ranges of MICs, MICs for 50 and 90% of the strains, and the percentage of susceptible strains are given by genus. For selected antimicrobial agents, the ranges of MICs are given by species in Table 2.

Glycopeptides. For all the strains tested, vancomycin MICs were ≥256 μg/ml, with no zone in the disk diffusion test. Teicoplanin and daptomycin, antimicrobial agents similar to vancomycin, were also tested. For one strain of *P. acidilactici*, the teicoplanin MIC was 64 μg/ml; for the others, the MICs were ≥128 μg/ml. All the strains tested were susceptible to daptomycin, for which the MICs were ≤0.25 μg/ml.

Beta-lactam agents. The MICs of penicillin for 96% of the strains tested (81 of 84) were in the range of 0.25 to 2.0 μg/ml. Ampicillin MICs ranged 1 to 2 concentrations higher. All strains had oxacillin zone diameters of 6 mm (i.e., no zone), including the single type strain of *L. dextranicum*, for which the MIC of penicillin was 0.03 μg/ml. For the cephalosporins tested, cephalothin was the most active overall, but cefuroxime, ceftizoxime, and cefotaxime were more active against the strains of *L. citreum* and several other isolates than was cephalothin. As with other gram-positive bacteria, these genera were less susceptible to ceftazidime than to other broad-spectrum cephalosporins. For all of the pediococci and the lactobacilli tested, imipenem MICs were ≤0.12 μg/ml, which distinguished them from the majority of the *Leuconostoc* spp. tested. Except for the single type strains of *L. paramesenteroides* and *L. dextranicum* and two of the unidentified *Leuconostoc* spp., all other imipenem MICs for the *Leuconostoc* spp. were ≥1.0 μg/ml. No β-lactamase was detected in any of the strains.

Macrolides. For all except two strains of pediococci, the erythromycin MICs were ≤0.06 μg/ml; for these two pediococci, the MICs were 4 and >128 μg/ml. The activities of roxithromycin and josamycin were similar. The strain of *P. acidilactici* that was highly resistant to erythromycin was also resistant to clindamycin (MIC = 32 μg/ml); however, the strain of *P. pentosaceus* for which the erythromycin MIC was 4 μg/ml was susceptible to clindamycin and could not be induced to exhibit clindamycin resistance. For three strains of *Leuconostoc* spp., the MICs of clindamycin were increased (0.12 to 2 μg/ml) and zone diameters were in the

TABLE 2. Range of MICs by species for selected antimicrobial agents

Organism (*n*)	Range of MICs (μg/ml)									
	Penicillin	Cephalothin	Cefuroxime	Ceftizoxime	Ceftriaxone	Imipenem	Erythromycin	Rifampin	Gentamicin	Ciprofloxacin
Leuconostoc mesenteroides (19)	0.5–1	2–16	8–32	8–128	8–64	2–8	≤0.015–0.06	0.5–8	≤0.25–0.5	1–4
L. citreum (12)	0.12–0.5	0.5–2	≤0.25–1	≤0.25–1	≤0.25–4	1–2	0.03–0.06	1–2	≤0.25	0.5–2
L. pseudomesenteroides (4)	0.12–0.5	0.5–4	0.5–16	≤0.25–16	1–16	1–8	0.06	1–2	≤0.25–0.5	2–4
L. lactis (3)	0.5	2–4	2–8	2–4	4–16	2–4	0.06	1–8	≤0.25–0.5	2
L. dextranicum (1)	0.03	0.12	0.5	0.5	1	0.12	≤0.015	0.06	≤0.25	1
L. paramesenteroides (1)	0.5	32	8	8	4	≤0.06	0.06	64	≤0.25	2
Leuconostoc species (7)	0.25–2	1–32	0.5–16	0.5–128	2–128	0.12–4	0.06	1–64	≤0.25–0.5	1–4
Pediococcus acidilactici (19)	0.5–1	2–16	4–16	4–32	4–16	≤0.06–0.12	≤0.015–>128	0.12–4	0.5–4	2–16
P. pentosaceus (5)	1–2	8–16	8–16	16–32	2–8	≤0.06–0.12	0.03–4	1–2	0.5–1	8–16
Lactobacillus confusus (11)	0.25–2	8–16	2–>128	4–>128	1–>128	≤0.06–0.12	0.06	16–32	≤0.25–1	1–2
Lactobacillus species (2)	0.25, 0.5	8, 16	16–>128	16–>128	8–64	≤0.06–0.12	0.03–0.06	16–32	≤0.25	1–2

resistant range, but the strains showed no increased resistance to erythromycin.

Aminoglycosides. Gentamicin was the most active of the four animoglycosides tested and had the greatest activity against the leuconostocs. For one strain of *P. acidilactici*, the streptomycin MIC was 2,000 μg/ml.

Miscellaneous agents. For the tetracyclines, minocycline was the most active, followed by doxycycline. Except for one strain of *L. citreum* for which the chloramphenicol MIC was 16 μg/ml, the chloramphenicol MICs were ≤8 μg/ml. However, 11 strains (9 *L. confusus*, 1 *Leuconostoc* sp., and 1 *L. citreum*) had zone diameters between 13 and 17 mm, indicating an intermediate susceptibility. Chloramphenicol acetyltransferase, however, was not detected in any of these strains, even after induction.

For all the lactobacilli tested, the MICs of rifampin were 16 to 32 μg/ml, and for all of the pediococci the MICs were ≤4 μg/ml. For 9 of the 47 *Leuconostoc* spp. (19%), the rifampin MICs were ≥8 μg/ml. For the majority (89%) of strains, ciprofloxacin MICs were in the range of 1 to 8 μg/ml, with higher MICs for the pediococci. Despite several MICs for ciprofloxacin that were in the range of 2 to 4 μg/ml, all the pediococci tested had no zone by disk diffusion. All of the leuconostocs and lactobacilli had zones ≥10 mm.

For four *Leuconostoc* strains, the sulfamethoxazole MICs were 64 to 128 μg/ml; for the remainder of strains tested, the MICs were ≥256 μg/ml. The vast majority of the leuconostocs and pediococci were susceptible to trimethoprim, whereas for only 3 of the 13 lactobacilli tested, the MICs were ≤8 μg/ml. None of the lactobacilli were susceptible to the trimethoprim-sulfamethoxazole combination; susceptibility varied for the other species tested.

Disk diffusion testing. Scatterplots for 8 of the 10 antimicrobial agents tested by disk diffusion are shown in Fig. 1. Results for vancomycin and oxacillin are discussed above. Discrepancies between the disk diffusion interpretation and the MIC category interpretation are assumed to be errors in the disk diffusion test and are summarized in Table 3. Only one (0.1%) very major error (susceptible by disk diffusion and resistant by MIC) occurred (with tetracycline) and 10 (1.5%) major errors (resistant by disk diffusion and susceptible by MIC) occurred, but the number of minor errors (intermediate by one of the tests) was very high (19.3%), with tetracycline and ciprofloxacin having the most.

DISCUSSION

Knowledge of the patterns of susceptibility of vancomycin-resistant gram-positive organisms should help physicians treat infections caused by these strains. Many of the antimicrobial agents that we tested did not have uniform activities against the three genera tested, so it appears that proper identification will help in the formulation of optimal antimicrobial regimens. Although for most of the strains tested penicillin MICs were in the moderately susceptible range as defined by the NCCLS for nonenterococcal streptococci, for three of the *Leuconostoc* strains tested (the type strain of *L. dextranicum* and one strain each of *L. citreum* and *L. pseudomesenteroides*) the MICs were in the susceptible range. Of the other agents, those with the best activities against all the strains tested were imipenem, chloramphenicol, erythromycin, clindamycin, gentamicin, and daptomycin.

For the agents tested in common, our results are similar to those of Buu-Hoi et al. (2), who tested only a small number of *Leuconostoc* spp. and employed an agar dilution method, using Mueller-Hinton agar supplemented with 5% blood, but incubated plates in an atmosphere of 10% CO_2. de la Maza et al. (6) have reported MICs for all three species, also using an agar dilution technique but incubating plates in 5 to 7% CO_2. For some organism-drug combinations, the MICs for 90% of the strains tested that de la Maza et al. reported (shown first in parentheses) were higher than ours (shown second in parentheses): *Lactobacillus* sp. with daptomycin (2 versus 0.25 μg/ml), clindamycin (0.5 versus 0.06 μg/ml), and gentamicin (8 versus 0.5 μg/ml) and *Leuconostoc* sp. with erythromycin (0.25 versus 0.06 μg/ml), clindamycin (1 versus 0.06 μg/ml), and gentamicin (4 versus 0.5 μg/ml). However, we did not find it necessary to incubate the broth microdilution plates in increased CO_2 as they did with their agar dilution plates, which might explain the discrepancies, since the agents listed above are know to be affected by either medium or pH differences caused by CO_2 incubation.

We noticed an interesting difference in patterns of two of the drugs tested that could help in differentiating the three genera. For these three vancomycin-resistant genera, all the *Pediococcus* strains had no zones with ciprofloxacin but the imipenem MICs were ≤0.12 μg/ml; for all the *Leuconostoc* strains, ciprofloxacin zones were ≥11 mm and, for the most, the imipenem MICs were ≥1.0 (except for the type strains of *L. dextranicum* and *L. paramesenteroides* and two of the seven *Leuconostoc* spp.); all the *Lactobacillus* strains had ciprofloxacin zones of ≥13 mm and the imipenem MICs for the strains were ≤0.12 μg/ml. Use of these characteristics could help in the early identification of these strains to the genus level; however, given the sometimes fickle nature of resistance patterns, the identification should be confirmed.

When the usefulness of a new procedure against a reference procedure is evaluated, Thornsberry (25) has suggested that overall agreement should be greater than 90%, with very major and major errors less than 5%. Sherris and Ryan (23) are even more stringent in their evaluation, suggesting that very major errors should be less than 1.5% and that overall errors should be less than 5% (i.e., overall agreement greater than 95%). In our study, when broth microdilution was compared with disk diffusion, the number of very major and major errors was 1.6% (Table 3) and the overall agreement was 80.7%. Although errors were the greatest for tetracycline and ciprofloxacin, none of the antimicrobial agents agreed more than 90% of the time. We thought that the reason for the high percentage of errors may be that many of the MICs fall into the intermediate category, but even for those antimicrobial agents with a low number of intermediate values (Table 3) the overall agreement does not improve. It may be that the number of disagreements could be lowered by changing the breakpoints for these organisms, but, for the time being, we do not recommend it. Even though there was only one very major error (with tetracycline, a drug that is an unlikely choice for treatment of infections caused by these organisms), we feel that precise definition of the susceptibility of these organisms is best determined by using an MIC test.

For all except three of the isolates tested, the penicillin MICs were in the moderately susceptible range (0.25 to 2 μg/ml) if breakpoints for nonenterococcal streptococci are used, whereas all the isolates would be characterized as moderately susceptible to penicillin (including the one isolate for which the penicillin MIC was 0.03 μg/ml) if breakpoints for enterococci are used. However, successful treatment with penicillin alone has been reported for several infections caused by these organisms, including the one case of meningitis (2, 4, 5, 12, 20). Unless it is shown clinically

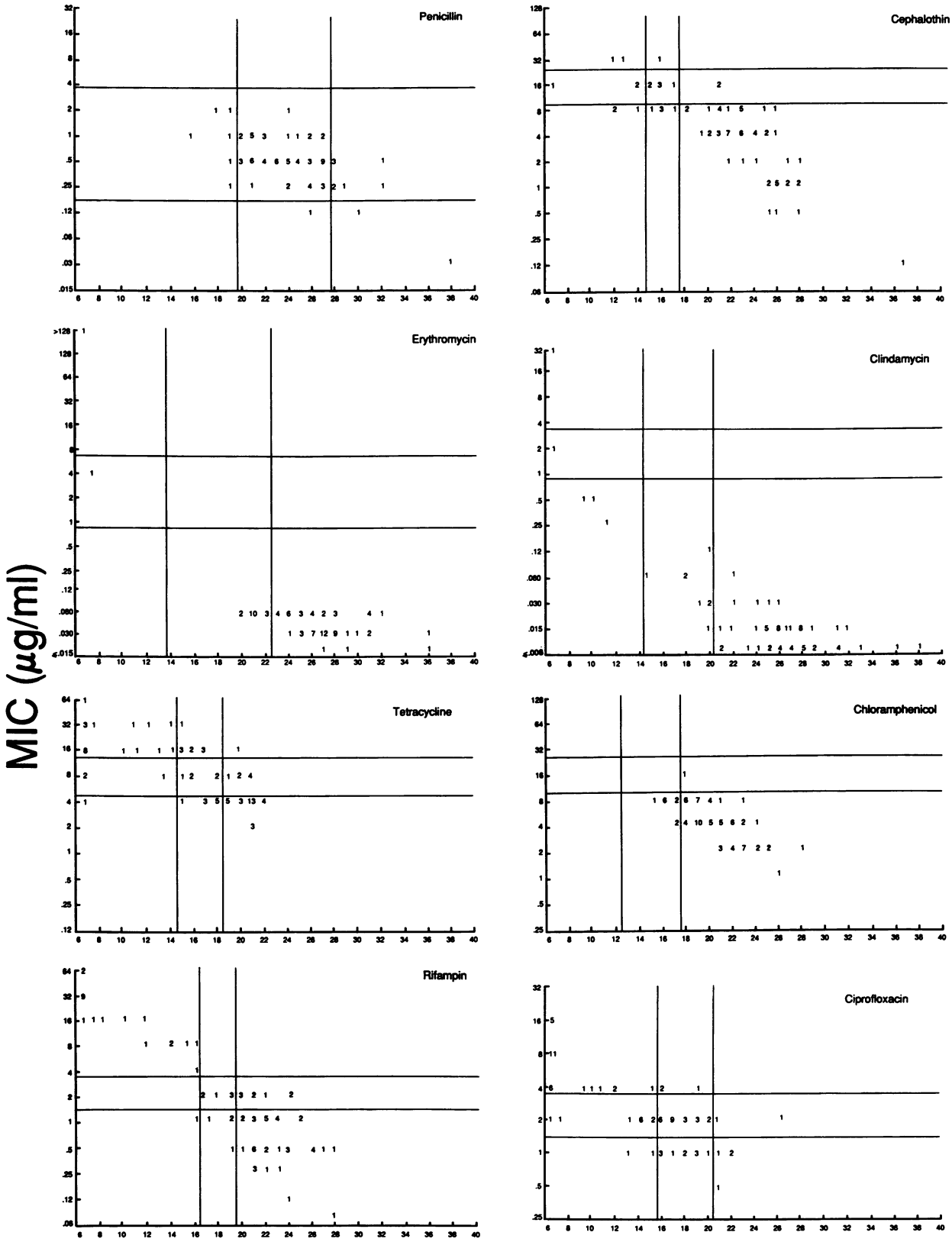

FIG. 1. Scatterplots of MICs and zone diameter after incubation in 5% CO_2. Breakpoints used are those recommended by the NCCLS (17). For penicillin they are the breakpoints for nonenterococcal streptococci.

TABLE 3. Errors of disk diffusion compared with broth microdilution MICs

Drug[a]	No. of errors (%)			% Overall agreement	% Intermediates[b] by:	
	Very major[c]	Major[d]	Minor[e]		MIC	Disk diffusion
Penicillin			15 (17.9)	82.1	96.4	83.3
Cephalothin		3 (3.6)	11 (13.3)	83.1	13.3	14.5
Tetracycline	1 (1.2)	1 (1.2)	28 (33.3)	64.3	17.9	27.4
Chloramphenicol			12 (14.3)	85.7	1.2	13.1
Erythromycin			16 (19.0)	81.0	1.2	0
Clindamycin		3 (3.6)	9 (10.7)	85.7	1.2	9.5
Rifampin		1 (1.2)	12 (14.5)	84.3	16.7	12.0
Ciprofloxacin		2 (2.4)	26 (31.3)	66.3	43.4	43.4
Total	1 (0.1)	10 (1.5)	129 (19.3)	80.7		

[a] Number of zone-MIC combinations was 84 for all drugs except ciprofloxacin, cephalothin, and rifampin, for which it was 83.
[b] Percentage of strains in intermediate category.
[c] Susceptible by disk diffusion, resistant by MIC.
[d] Resistant by disk diffusion, susceptible by MIC.
[e] Intermediate for one test, susceptible or resistant for the other.

that it is better to use the more stringent enterococcal breakpoints, we prefer to use the nonenterococcal breakpoints which still classify the majority of strains as moderately susceptible.

Given the numerous reports that have been published since 1988 (5, 10, 13, 20, 21, 27), it appears indisputable that nonenterococcal vancomycin-resistant, gram-positive organisms should now be considered clinically significant when isolated in the proper circumstances. But because of problems in recognition and identification of this group, many of these strains may have been overlooked or misidentified. The use of vancomycin resistance to separate these isolates from what may have previously been identified as alpha-hemolytic streptococci (7) may help clinical laboratories to get a better idea of the true prevalence and importance of this group. Methods for distinguishing the different genera (7) will also help us to further our understanding of these organisms.

LITERATURE CITED

1. **Bayer, A. S., A. W. Chow, D. Betts, and L. B. Guze.** 1978. Lactobacillemia—report of nine cases. Important clinical and therapeutic considerations. Am. J. Med. **64**:808–813.
2. **Buu-Hoi, A., C. Branger, and J. F. Acar.** 1985. Vancomycin-resistant streptococci or *Leuconostoc* sp. Antimicrob. Agents Chemother. **28**:458–460.
3. **Colman, G., and A. Efstratiou.** 1987. Vancomycin-resistant leuconostocs, lactobacilli, and now pediococci. J. Hosp. Infect. **10**:1–3.
4. **Coovadia, Y. M., Z. Solwa, and J. van den Ende.** 1987. Meningitis caused by vancomycin-resistant *Leuconostoc* sp. J. Clin. Microbiol. **25**:1784–1785.
5. **Coovadia, Y. M., Z. Solwa, and J. van den Ende.** 1988. Potential pathogenicity of Leuconostoc. Lancet **i**:306.
6. **de la Maza, L., K. L. Ruoff, and M. J. Ferraro.** 1989. In vitro activities of daptomycin and other antimicrobial agents against vancomycin-resistant gram-positive cocci. Antimicrob. Agents Chemother. **33**:1383–1384.
7. **Facklam, R., D. Hollis, and M. D. Collins.** 1989. Identification of gram-positive coccal and coccobacillary vancomycin-resistant bacteria. J. Clin. Microbiol. **27**:724–730.
8. **Golledge, C.** 1988. Vancomycin resistant lactobacilli. J. Hosp. Infect. **11**:292.
9. **Haley, R. W., A. W. Hightower, R. F. Khabbaz, C. Thornsberry, W. J. Martone, J. R. Allen, and J. M. Hughes.** 1982. The emergence of methicillin-resistant *Staphylococcus aureus* infections in United States hospitals. Ann. Intern. Med. **97**:297–308.
10. **Hardy, S., K. L. Ruoff, E. A. Catlin, and J. I. Santos.** 1988. Catheter-associated infection with a vancomycin-resistant gram-positive coccus of the *Leuconostoc sp.* Pediatr. Infect. Dis. J. **7**:519–520.
11. **Holliman, R. E., and G. P. Bone.** 1988. Vancomycin resistance of clinical isolates of lactobacilli. J. Infect. **16**:279–283.
12. **Horowitz, H. W., S. Handwerger, K. G. van Horn, and G. P. Wormser.** 1987. Leuconostoc, an emerging pathogen. Lancet **ii**:1329–1330.
13. **Isenberg, H. D., E. M. Vellozzi, J. Shapiro, and L. G. Rubin.** 1988. Clinical laboratory challenges in the recognition of *Leuconostoc* spp. J. Clin. Microbiol. **26**:479–483.
14. **Kaplan, A. H., P. H. Gilligan, and R. R. Facklam.** 1988. Recovery of resistant enterococci during vancomycin prophylaxis. J. Clin. Microbiol. **26**:1216–1218.
15. **LeClerq, R., E. Derlot, J. Duval, and P. Courvalin.** 1988. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. N. Engl. J. Med. **319**:157–161.
16. **Lutticken, R., and G. Kunstmann.** 1988. Vancomycin-resistant Streptococcaceae from clinical material. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A **267**:379–382.
17. **National Committee for Clinical Laboratory Standards.** 1988. Performance standards for antimicrobial disk susceptibility tests, M2-T4. National Committee for Clinical Laboratory Standards, Villanova, Pa.
18. **National Committee for Clinical Laboratory Standards.** 1988. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, M7-T2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
19. **Orberg, P. K., and W. E. Sandine.** 1984. Common occurrence of plasmid DNA and vancomycin resistance in *Leuconostoc* spp. Appl. Environ. Microbiol. **48**:1129–1133.
20. **Rubin, L. G., E. Vellozzi, J. Shapiro, and H. D. Isenberg.** 1988. Infections with vancomycin-resistant "streptococci" due to Leuconostoc species. J. Infect. Dis. **157**:216.
21. **Ruoff, K. L., D. R. Kuritzkes, J. S. Wolfson, and M. J. Ferraro.** 1988. Vancomycin-resistant gram-positive bacteria isolated from human sources. J. Clin. Microbiol. **26**:2064–2068.
22. **Schwalbe, R. S., J. T. Stapleton, and P. H. Gilligan.** 1987. Emergence of vancomycin resistance in coagulase-negative staphylococci. N. Engl. J. Med. **316**:927–931.
23. **Sherris, J. C., and K. J. Ryan.** 1982. Evaluation of automated and rapid methods, p. 1–5. *In* R. C. Tilton (ed.), Rapid methods and automation in microbiology. American Society for Microbiology, Washington, D.C.
24. **Shlaes, D. M., J. Marino, and M. R. Jacobs.** 1984. Infection caused by vancomycin-resistant *Streptococcus sanguis* II. Antimicrob. Agents Chemother. **25**:527–528.
25. **Thornsberry, C.** 1985. Automated procedures for antimicrobial susceptibility tests, p. 1015–1018. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
26. **Uttley, A. H. C., C. H. Collins, J. Naidoo, and R. C. George.** 1988. Vancomycin-resistant enterococci. Lancet **ii**:57–58.
27. **Wenocur, H. S., M. A. Smith, E. M. Vellozzi, J. Shapiro, and H. D. Isenberg.** 1988. Odontogenic infection secondary to *Leuconostoc* species. J. Clin. Microbiol. **26**:1893–1894.